SOP: 5-2
Standard Operating Procedure
Litter and Soil Sample Compositing

Revision: 3*
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Prepared: Todd Burgesser Review: Kim Zilis

Approved: Roger L. Olsen Date Approved: 2/06/07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for compositing of soil and poultry litter samples from the Illinois River watershed of eastern Oklahoma and western Arkansas. This will include handling, mixing, and shipment of soil and litter samples.

2.0 Handling and Compositing of Soil and Litter Samples

All individual soil samples from each sub-location will be placed in individual plastic bags (double bagged), packed in a cooler with blue ice and shipped over night under chain-of-custody to the CDM processing laboratory in Denver, Colorado. The sample number will be located between the inner and outer plastic bag. Each sampling area (up to four sampling areas per LAL) will have 60 individual samples (20 sub-sample locations x 3 sample depths = 60 samples). All samples will be received by the CDM processing laboratory for compositing. Each of the 20 sub-samples will be composited into one homogeneous sample using the protocol described below.

Litter samples will be received by the CDM processing laboratory under chain-of custody in a 5-gallon bucket. The litter sample will be contained in a plastic bag inside of the 5-gallon bucket will be closed with a tie. A unique sample number will be written on the outside of the bucket.

Upon receipt of the samples, the cooler/bucket temperature will be measured using a NIST traceable thermometer. The samples soil will then be removed from the cooler and checked against the chain-of-custody to ensure that all samples have been received.

The twenty sub-samples associated with the individual sample depths or the entire litter sample will be poured into a stainless steel bowl or 2.5-gallon bucket ready for mixing. All equipment will be decontaminated/sterilized with laboratory grade distilled water and 10 percent bleach (see procedure below).

EXHIBIT D

2.1 Mixing of Soil Samples

- All health and safety protocol will be followed as described in the Health and Safety Plan for the Illinois River Basin Project. This is includes wearing nitrile gloves and processing soil in the hood.
- All feathers, rocks, twigs, debris and vegetation will be removed before sieving and mixing.
- Mixing will be accomplished using a disposable, plastic sampling scoop or a decontaminated stainless steel spoon.
- All clods over 0.5 inches in diameter will be disaggregated into smaller particles by hand or the use of a decontaminated stainless steel spoon or mortar.
- If the moisture content is too high to allow homogenization or disaggregation of the particles, the sample will be placed in steel drying pan and air dried over night.
- The sample will be hand mixed using the plastic scoop or stainless steel spoon for at least five minutes or until particles are uniform in size.
- If a plastic bucket is used, the bucket will then be sealed and inverted or rotated at least 10 times.
- After mixing, the sample will be sieved to remove particles sizes of greater than 2 mm using a decontaminated US Sieve no. 10 (gravel size particles will be removed).
- Each fraction (greater than 2 mm and less than 2 mm) will be weighted. The less than 2 mm fraction will be placed in a plate grinder and reduced in size to 0.074 mm (US sieve no. 200, very fine sand).
- The ground sample will be split using a riffle splitter and sent to the various laboratories (see splitting procedure in section 1.3.1, Duplicate Samples).

2.2 Mixing of Litter Samples

The same procedure as described above for the soil will be used for the litter. However, grinding may not be necessary if the litter can be sieved directly through a US sieve no. 200.

2.3 Laboratory QA/QC Samples (Soil)

Laboratory QA/QC samples may consist of duplicate samples, decontamination blanks, and blind standards. The following describes each type of QA/QC sample.

2.3.1 Duplicate Samples (created at the soil processing lab)

After sample mixing, sieving and grinding, two split samples will be collected. The sub-sample splits should be collected using a nonbiased riffle splitter. The sample is poured through the riffle splitter and into the decontaminated collection pans. The amount of soil or litter contained by the sample container shall be sufficient for the chemical and physical analyses to be conducted.

2.3.2 Blind Standards

A blind standard of a certified reference soil will be sent to the analytical laboratory for approximately every 50 samples send to the laboratory. The blind standard will be sent by the CDM soil processing lab. Blind standards will be for metals, arsenic, and phosphorus.

2.3.3 Decontamination Blanks

A sample of the final decontamination rinsate will be collected and forwarded to the analytical laboratory for analysis. The decontamination rinsate blank will be generated in the CDM processing laboratory using a final rinse of laboratory grade distilled water. All parameters will be analyzed.

3.0 Shipment of Samples to the Analytical Laboratory

- Once placed in sampling containers (plastic bags or jars), samples will be held at 4° C on blue ice (sealed in plastic bags) within insulated protective containers.
- If possible, samples will be shipped immediately after compositing via overnight shipment to the analytical laboratory.
- After compositing, samples should not be held more than 24 hours before shipment.
- Samples will be sent to the laboratory under a Chain-of-Custody.
- A custody seal will be place on the outside of the cooler between the lid and the body of the cooler. The custody seal will be signed.
- The Chain-of-Custody will be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.

4.0 Decontamination of Processing Equipment

All nondisposable equipment (bowls, sieves, spoons, and grinders) will be decontaminated/sterilized after each composite sample is created. Decontamination will include washing with phosphate free water followed by rinsing with laboratory grade distilled water. A final rinse of 10 percent bleach will be performed. The equipment will be air dried.

5.0 List of Analytes and Bottle Requirements

5.1 Analytical Parameters

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 1 provides the parameters and analytical methods for the short list and Table 2 provides the parameters and analytical methods for the long list.

Litter samples will be analyzed for Table 2 parameters.

Table 1: Short List Parameters - Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Nitrogen	Kjeldahl, modified
Total Aluminum (Al)	EPA SW-3050/6010/6020
Total Phosphorous (P)	EPA SW-3050/6010/6020
Total Arsenic (As)	EPA SW-3050/6010/6020
Total Copper (Cu)	EPA SW-3050/6010/6020
Total Zinc (Zn)	EPA SW-3050/6010/6020

Table 2: Long List Parameters – Manure and Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Texture (% sand, silt and clay)*	Hydrometer ASTM-D422
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Phosphorous (P)	EPA SW-3050/6020
Mehlich-III Phosphorous (Mehlich-III P)	Mehlich III (ICP)

Soluble Phosphorous	Water 1:10, Bull.396, pg 17
Soluble nitrate	Water 1:10
Total Nitrogen	Kjeldahl, modified
Soluble ammonium	Water 1:10
Soluble sulfate	Water 1:10
Soluble chloride	Water 1:10
TAL Metals	EPA SW-3050/6020
Total Molybdenum (Mo)	EPA SW-3050/6020
Bacteria:	
Total coliform	SM-9221B
enterococcus	SM-9230B
Fecal coliform	SM-9221E
e-coli	SM-9221F
staphylococcus	BAM12
campylobacter	BAM7
salmonella	BAM5
17β-estradiol, estrone, estriol	LC-MS-MS

*split before sieving and grinding

5.2 Bottle Requirements

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 3 provides the parameters, bottle requirement and laboratory for the short list and Table 4 provides the parameters, bottle requirement and laboratory for the long list.

Litter samples will be analyzed for Table 4 parameters.

Table 3: Short List Parameters - Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Total Aluminum (Al)	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Total Arsenic (As)	1 quart glass	A&L
Total Copper (Cu)	1 quart glass	A&L
Total Zinc (Zn)	1 quart glass	A&L

Note: 1 bottle for all of the above analysis

Table 4: Long List Parameters – Manure and Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Texture (% sand, silt and clay)*	1 quart glass (separate from the other bottles)	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Mehlich-III Phosphorous (Mehlich-III P)	1 quart glass	A&L
Soluble Phosphorous	1 quart glass	A&L
Soluble nitrate	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Soluble ammonium	1 quart glass	A&L
Soluble sulfate	1 quart glass	A&L
Soluble chloride	1 quart glass	A&L
TAL Metals	1 quart glass	A&L
Total Molybdenum (Mo)	1 quart glass	A&L
Bacteria	1 - 250 mL plastic (sterilized) or 1-8 oz. Whirl bag	EML
PCR	1-8 oz. Whirl bag	ISU
17β-estradiol, estrone, estriol	1 – 4oz. glass	GEL

*split before sieving and grinding

6.0 Analytical Laboratories

Bottles for estrogen metabolites (all samples) will be shipped to:

General Engineering Laboratories, LLC
201 Pine Ridge Road, Unit 5
Golden, CO 80403
Contact: Paul Winkler, 720-253-3093
Paul.winkler@gel.com

Bottles for nutrients, metals, etc (all samples) will be shipped to:

A&L Analytical Laboratories, Inc.
2790 Whitten Rd.
Memphis, TN 38133
Contact: Scott McKee, 800-264-4522
smckee@allabs.com

Bottles for bacteria analyses from soil and litter will be shipped to:

Environmental Microbiology Laboratory
1150 Bayhill Drive, Suite 100
San Bruno, CA 94066
Contact: Megan S. Tatreau, 858-268-2770
mtatreau@emlab.com

Bottles for PCR will be shipped to:

Idaho State University
Department of Biological Sciences-MRCF
Attn: Erin O'Leary-Jepsen
640 Memorial Drive
Pocatello, ID 83209-8007
Contact: Erin O'Leary-Jepsen, 208-282-4890

7.0 Documentation

Bound laboratory logbooks should be used for the maintenance of field records. All aspects of sample compositing and handling as well as visual observations will be documented in the field logbooks. Supplemental information may be documented on the field data sheets provided. All entries in laboratory logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

8.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 2: 02/09/06

Revision 1: 05/10/05